Review Article: Peritoneal Washing Cytology

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Running Title: Peritoneal Washing Cytology

Abstract

Peritoneal washing cytology (PWC) is a useful indicator of ovarian surface involvement and peritoneal dissemination by ovarian tumours. It may identify subclinical peritoneal spread and thus provide valuable staging and prognostic information, particularly for non-serous ovarian tumours. The role of PWC as a prognostic indicator for endometrial carcinoma is less clear, due in part to the questionable significance of identifying endometrial tumour cells in the peritoneum.

Detection of metastatic carcinoma in PWC is based on recognition of non-mesothelial cell characteristics, however a number of conditions such as reactive mesothelial cells, endometriosis and endosalpingiosis may mimic this appearance. Cells from these conditions may have a similar presentation in PWC to that of serous borderline tumours and low grade serous carcinoma. The presence of cilia, lack of single atypical cells, prominent cytoplasmic vacuolation, marked nuclear atypia or two distinct cell populations are features favouring a benign process. Attention to these features along with close correlation with clinical history and the results of surgical pathology should help avoid errors. Additional assistance may be provided by the use of cell blocks and special stains.

Keywords: Peritoneal Washing, Peritoneum, Cytology, Gynaecological Malignancy, Immunocytochemistry

Introduction

Peritoneal dissemination by gynaecological malignancies, particularly ovarian tumours, often manifests in malignant ascites. Microscopic peritoneal seeding with tumour cells predates the formation of ascites and its detection by peritoneal washing cytology (PWC) may provide valuable staging and prognostic information. Identification of patients with occult (microscopic) peritoneal dissemination may be useful in defining a group of patients that would benefit from different treatment. The technique of intraoperative peritoneal washing cytology was introduced in 1956 by Keetle and Elkin [1]. In 1975 the International Federation of Gynecologists & Obstetricians (FIGO) incorporated results of PWC into the staging classification for ovarian carcinoma and in 1989 for endometrial carcinoma. The technique may also be useful in identifying recurrent or persistent cancer and has been applied to a variety of non-gynaecological cancers.

This review discusses the role of PWC and its morphological interpretation for the most common malignancies encountered. Pitfalls in diagnosis and adjunctive techniques are also reviewed.

Preparation and Specimen Adequacy

Specimen adequacy in PWC is poorly defined. DeMay[2] points out that the majority of published series contain no cases reported as unsatisfactory while a few have surprisingly high rates. Low cellularity may be a cause of false negative results [3].

Peritoneal washing specimens are frequently heavily contaminated with blood and methods to concentrate diagnostic cells are essential. Several methods, including Carnoy's fixation and gradient density centrifugation have been reported. Mulvaney[4] used Ficoll gradient density centrifugation to achieve this and reported only 1.5% (9/613) of specimens inadequate for interpretation.

In our laboratory we find blood removal is almost always required for PW specimens. Specimens are centrifuged, the supernatant discarded and two direct smears are prepared. One is fixed in 95% alcohol for Pap staining and the other air-dried for rapid Giemsa staining. If the specimen is bloody, the remaining pellet is then resuspended in a Hanks/methanol solution (HMS: 1 part methanol; 2 parts Hank's balanced salt solution; 3 parts distilled water) and agitated for 10 minutes. This step is highly effective at lysing the red blood cells without significantly altering cellular arrangements or morphology. The solution is then centrifuged again and if a pellet is visible then a direct smear is prepared and alcohol fixed. If only a scanty pellet remains, the material is processed as a cytospin for Pap staining. This processing allows

evaluation of the specimen in its ‘native’ state as well as concentrating diagnostic cells from a large proportion of the submitted sample. In our experience it results in a very low proportion of specimens reported as ‘blood only’.

Applications

Although positive PWC is useful for documenting the presence of malignant cells in the peritoneum, this is not always of prognostic significance. Positive PWC is generally seen in patients with a poor prognosis, however for some primary sites this is not an independent factor, but rather is associated with other prognostic indicators such as lymph node involvement and deep stromal invasion. Additionally, not all free cancer cells in the peritoneum survive and establish intraperitoneal metastases.

Benign Findings

Peritoneal washing specimens are generally highly cellular with mesothelial cells arranged in monolayered sheets. The cells are regularly spaced with a moderate amount of cytoplasm, usually forming pavement-like sheets (Figure 1). Nuclei may vary considerably in size from sheet to sheet but are usually locally uniform with fine, even chromatin and small nucleoli (Figure 2). Nuclear longitudinal grooves and sometimes-folded nuclear membranes may be seen (Figure 3). The latter are said to be more common in mid-cycle[2]. The most common pattern is numerous, sometimes quite large, sheets of

mesothelial cells with minimal leucocytes and single cells. Variations on this pattern include increased numbers of single cells, including macrophages, leucocytes and mesothelial cells, and, in reactive conditions, the presence of smaller, rounded groups with greater depth of focus.

Other benign findings include skeletal muscle fragments and collagen balls. So-called collagen balls (Figure 4) are spheroids of collagen surrounded by a single layer of mesothelial cells[5]. In Papanicolaou stained preparations they appear as distinctive aqua coloured balls of homogenous material. By focusing through the ball it is possible to identify its rounded nature and the single layer of mesothelial cells with bland nuclei. Examination of surgical specimens suggests that collagen balls originate from minute papillary stromal projections on the surface of the ovary that are covered with mesothelium[5]. Wojcik and Naylor[5] reported finding collagen balls in 4.5% of 418 peritoneal washing specimens. In our experience they are seen much more frequently. Review of 80 consecutive specimens identified collagen in balls in 48%, occurring in almost half of both benign and malignant specimens. This higher frequency may be the result of the thorough sampling of the specimen provided by our preparation method. Although they are listed as a possible cause for false positive diagnoses by some authors[5,6], collagen balls are usually an easily recognised incidental benign finding.

Benign Gynaecological Disease

Although PWC may identify occult carcinoma during surgery for benign disease the incidence of this is very low. Several large series have failed to find any cases with positive PWC amongst patients with benign gynaecological disease [7,8,9].

Ovarian Tumours

For ovarian tumours positive PWC is a sensitive indicator of ovarian surface involvement and peritoneal dissemination and will detect a high proportion of patients with subclinical intraperitoneal extension[10]. The technique provides wider sampling of the peritoneum than random staging biopsies. Several authors have reported positive PWC in cases of ovarian borderline tumours of low malignant potential (LMP) without other evidence of peritoneal implants or ovarian surface involvement[4,10].

Peritoneal washing cytology plays a valuable role in the staging of ovarian tumours. Four year survival of patients with ovarian carcinoma has been shown to be significantly different depending on PWC regardless of other parameters[11]. Mulvaney[4] reported 63% (5/8) of borderline ovarian tumours and 50% (3/6) of non-serous ovarian malignancies were upstaged as a result of PWC results. However for serous carcinomas, only 7% (1/14) were upstaged on PWC, probably due to the clinically obvious dissemination of tumours on presentation.

Morphologically, ovarian epithelial tumours show a spectrum from benign cystadenomas through low malignant potential (borderline) tumours to malignant adenocarcinomas. Borderline tumours constitute about 10-15% of serous tumours and histologically show epithelial proliferation with stratification and tufting but lack destructive stromal invasion. About a third are associated with peritoneal implants, both invasive and non-invasive[12]. Borderline tumours have a better prognosis and generally are managed more conservatively and their distinction from low grade adenocarcinomas in PW specimens may therefore be important.

Borderline tumours tend to present in PW specimens as large, smooth bordered, papillary fragments composed of small uniform cells with high N/C ratio and inconspicuous nucleoli (Figure 5). Mitotic figures and cytoplasmic vacuoles are infrequent. Psammoma bodies may be seen in the PWC of half to two thirds of cases[4,13,14,15].

By contrast, the PWC of invasive ovarian carcinoma is characterised by smaller, less cohesive papillary fragments of cells with irregular borders. Individual cells are relatively larger and more pleomorphic with a low N/C ratio and abundant cytoplasmic vacuoles (Figure 6). In general PWC preparations are more cellular than seen in borderline tumours with frequent

single cells and mitoses. Adenocarcinomas are also more likely to be anueploid[14].

Although these general morphological differences exist and some studies have suggested that a distinction between borderline serous tumours and frank adenocarcinomas is possible[15], most studies of low grade carcinoma have concluded that in practice it is not possible to separate them from ovarian LMP tumours in PWC due to the overlap in features[10,12,14,16]. Correlation with histology results from surgical specimens is therefore an essential step.

Cytological typing of ovarian tumours is not usually required, as surgical specimens provide more reliable classification. Serous, mucinous and endometrioid tumours all generally show typical cytological features of adenocarcinoma. Although some features are more commonly expressed in certain tumours, such as psammoma bodies in serous tumours and mucin in mucinous tumours, there are no specific features[17]. Clear cell carcinomas are somewhat distinctive cytologically, usually showing single cells and sheets of cells with abundant finely vacuolated cytoplasm and round nuclei with prominent nucleoli (Figure 7). The cells may form small to medium-sized, rounded groups of cells surrounding metachromatic interluminal material[4,16]. In mixed Mullerian tumours it is usually the epidermal component that is present in PWC specimens. Germ cell tumours are usually easily recognised as

malignant, although some, such as yolk sac tumours, may closely resemble adenocarcinoma[18].

Endometrial Tumours

Unlike ovarian carcinoma, the role of PWC for endometrial carcinoma is not as clear cut. There is a significant increase in the incidence of abnormal PW in advanced stage disease (about 30% positive for stage III/IV compared with around 10% for stage I). However in advanced disease positive PWC may simply be a manifestation of extrauterine spread rather than an independent poor prognostic factor. It is in cases of early stage (I) disease where positive PWC could be of most prognostic importance. Unfortunately for Stage I endometrial carcinoma there are inconsistent results reported. Some series have shown significantly better survival rates if PWC is negative[1,19,20,21,22], however others have found no correlation of other histological parameters with positive PWC or with poorer survival[23,24]. Yet again, some series have reported correlation of positive PWC with some histological parameters but not with outcome[25,26,27]. These studies found PWC to be correlated with depth myometrial invasion, histological grade, cervical involvement and vascular channel involvement but not histologic subtypes, grade, adnexal or lymph node involvement.

In part this lack of clear-cut significance may be related to the mode of tumour cell spread from endometrial tumours to the peritoneum and to the variable

implications of tumour cells in the peritoneum. In the case of endometrial cancer, malignant cells identified in the peritoneum may not necessarily equal established metastatic disease.

The methods by which malignant cells reach the peritoneum is also of interest. In early stage disease where the tumour is otherwise confined to the uterus, the fallopian tube represents the most likely conduit. Menczer et al[28] reported positive tubal cytology in 22% of patients with endometrial carcinoma of all stages but positive PWC in only 8.5%. Seventy-five percent of patients with positive PWC had negative tubal cytology. In a study of fallopian tube washings, Mulvaney et al[4] found positive fallopian tube cytology with positive PWC in 72%. Metastasis via the lymphatics and haematogenous spread account for some peritoneal seeding as evidenced by cases of endometrial carcinoma with a history of BSO that develop positive PWC[29]. Lymphatic spread, as well as release of tumour cells from areas of serosal invasion has also been proposed as a method of peritoneal spread for gastric[30] and pancreatic cancer[31]. However, although some studies have found a correlation between positive PWC and vascular invasion for endometrial cancer[26,32], others have not[27]. Transtubal retrograde dissemination, for example during hysteroscopy, may be responsible for the presence of malignant cells in some cases of early stage endometrial carcinoma that are otherwise confined to the uterus. Sagawa et al[33] compared peritoneal cytology pre- and posthysterosopy and concluded that hysteroscopy resulted in 12.5% of cases

having positive PWC. Sonada et al[34] reported on 377 cases of Grade I/II endometrial carcinoma with no extrauterine spread. They found significantly more cases (independent of other prognostic factors) had positive PWC following laproscopic assisted vaginal hysterectomy (10%) than patients undergoing TAH (2.8%).

Some authors have attempted to correlate the morphology of tumour cells identified in PWC with outcome. Yanoh et al[35] concluded that cases with no evidence seeding and good outcome generally were less cellular; had less isolated cells and less groups with scalloped edges (Figure 8). Many of the cases that failed to develop macroscopic peritoneal disease showed sparse small 3-dimensional groups of cells with a morphology similar to that seen in endometrial carcinoma exfoliating and collected in Pap smears. A predominance of isolated cells in large numbers is also correlated with poor outcome in gastric[36] and pancreatic[31] carcinoma.

Cervical Tumours

PWC is of limited value for cervical carcinoma because peritoneal involvement generally occurs relatively late in the development of these tumours, with deep stromal and vascular involvement of more significance[11]. Mulvaney[37] reported several cases of cervical carcinoma that were upstaged as a result of positive PWC, however these patients had nodal metastases and their treatment was not altered. Adenocarcinomas of the cervix are more likely

to yield positive PWC than squamous tumours[37,38], as are recurrent cervical carcinoma cases[3]. SCC is rarely identified in PW specimens and most commonly presents as poorly differentiated malignant cells[38] .

Less Common Tumours

As well as carcinomas metastatic to the ovary, such as breast and colonic adenocarcinomas, fallopian tube cancer and primary peritoneal carcinoma are occasionally encountered in PWC. Serous papillary neoplasms may arise in the ovary, fallopian tube, endometrium and primary to the peritoneum. It is not easy to distinguish between the tumours on the basis of immunocytochemical profile[39] or cytomorphology- all have in common the features of single cells and three dimensional groups of cells with round nuclei, single nucleoli, abundant, often vacuolated cytoplasm and sometimes psamomma bodies[40].

Non-Gynaecological Malignancies

For gastric cancer, PWC provides clinically useful information. Positive PWC is associated with poor outcome for gastric cancer patients[41,42]. Patients with otherwise resectable gastric cancer and positive PWC have a survival comparable to stage IV tumours, even in the absence of macroscopic peritoneal disease and despite resection of all gross disease[41,42].

In pancreatic cancer, positive PWC is associated with advanced disease and may identify patients with occult peritoneal disease but is not an independent

risk factor[31,43]. Cases of long term survival, even with positive PWC have been reported and it is therefore not considered an indicator for non-resection[31]. For colonic cancer[44] and cholangiocarcinoma[45] positive PWC is not an independent prognostic indicator.

Pitfalls

Interpretive difficulties arise when a population of cells is present that appears different to the usual presentation of flat sheets of mesothelial cells. In addition to metastatic tumour, this may result from benign epithelium occurring in the peritoneum, such as Mullerian inclusions and fallopian tube epithelium, cells being spilt from ruptured cysts or due to reactive mesothelial changes. Benign reactive changes may be encountered in association with adhesion formation, pelvic inflammatory disease, endometriosis, ectopic pregnancy, adnexal infarction due to torsion, tubal abcess and hydrosalpinx.

Mesothelial Cells

Mesothelial cells, in all their various guises, pose the most common problems in PWC. In most cases the regular sheet-like arrangement of mesothelial cells that is characteristic of PWC makes their identification and discrimination from carcinoma easier than in effusion specimens where mesothelial cells are typically more rounded and the groupings more three dimensional. However large folded sheets of mesothelial cells in PW can sometimes mimic the papillary arrangements of carcinoma. Careful examination usually reveals the

flat, single-cell layering characteristic of benign mesothelial sheets. The often-marked variation in nuclear size amongst different groups of mesothelial cells in the same preparation may also contribute to their misinterpretation. However the cohesiveness, regular spacing and local uniformity of nuclei are useful in correctly identifying their benign nature.

Reactive mesothelial cells may be encountered in PW specimens, but more commonly in peritoneal effusions. They result of a wide variety of conditions, such as inflammatory conditions, endometriosis, ectopic pregnancy, tubo-ovarian abcess, pelvic tumour, chemo- and radiotherapy and cirrhosis[16,46,47]. Tight clusters of mesothelial cells may mimic low grade and borderline tumours with smooth edged groups of cells with tightly packed nuclei and high N/C ratios (Figure 9). Identification of a predominantly monolayered arrangement (Figure 10), fine, even chromatin and a gradation of cellular change from cells with typical mesothelial characteristics to those with reactive features[46] are helpful in recognising reactive change. Nevertheless, some cases demonstrate marked changes including prominent nucleoli and cells groups with considerable depth of focus and sometimes psammoma bodies. This can make discrimination from borderline and low grade tumours difficult (Figure 11). In some of these cases cell block preparations, immunocytochemistry or awaiting the results of surgical specimens are necessary to reliably identify the cell type. It should be emphasised that

psammoma bodies may be seen in a variety of benign conditions as well as borderline, invasive, and non-gynaecological tumours.

Endometriosis

The presence of endometrial glandular and stromal cells together with haemosiderophages is said to be diagnostic of endometriosis[2]. However endometrial cells and haemosiderophages are seen together in only about 30% of patients with laporoscopically confirmed endometriosis[48]. Haemosiderin-laden macrophages alone are seen in about another third of patients with confirmed pelvic endometriosis. Although they are a non-specific finding that may be seen in any condition associated with leakage of blood into the peritoneum, in young patients lacking other causes for haemorrhage, haemosiderophages are highly suggestive of endometriosis[48].

Endometrial cells are difficult to reliably identify, as they may be similar in presentation to mesothelial cells or lymphoid cells[48]. Perhaps the most reliable method of identifying them is in cell block preparations where the epithelial and stromal elements may be seen[6].

The most common difficulty caused by endometriosis is the presence of reactive mesothelial cells[2,3,16]. These may form large aggregates (Figures 12 & 13), sometimes with calcific concretions, mimicking serous tumours. Consideration of clinical history and observation of the mesothelial

characteristics of these groupings, such as limited depth of focus and uniformity of nuclear features and spacing, together with haemosiderophages are necessary to avoid false positive diagnoses.

Endosalpingiosis

Endosalpingiosis (Mullerian inclusions) is characterised by ectopic benign epithelium identical to fallopian tube epithelium. Endosalpingiosis is frequently multicentric, most commonly occuring in the pelvic peritoneum, lymph nodes and omentum and is thought to arise from a metaplastic process of the coelomic epithelum.

It is a potential cause for false positive diagnoses[3,49,50] as cytologically the cells from endosalpingioisis may present as tightly cohesive papillary fragments of cells that contrast with benign mesothelial cells. The cell aggregates are most commonly tubular or papillary and often surround psammoma bodies (Figures 14 & 15). Free psammoma bodies may be seen but single cells are usually lacking. Like reactive mesothelial cells, although the cells may have an increased N/C ratio and more prominent nucleoli, the epithelial groupings are usually single-layered with uniform, well spaced nuclei. In some cases cilia may be identified (Figure 16). The presence of more than mild to moderate nuclear atypia, complex branching architecture, single atypical cells or mitotic activity should weigh against a diagnosis of endosalpingiosis[49,50] .

Cell Blocks and Special Stains

Cell blocks provide a useful adjunct to cytological preparations of PWC[6]. They allow additional sampling of the specimen, assessment of microarchitecture and provide the optimal preparations for the performance of cytochemical and immunocytochemical (ICC) stains[51]. Cell blocks also provide a means for longer term 'storage' of material.

Several studies have reported improvements in sensitivity for detection malignant cells in PWC in gastric[52,53] and pancreatic[54,55] cancer by using ICC to a range of carcinoma markers, including B72.3, CEA and CA 19-9. The same markers for metastatic carcinoma that are of value in effusion cytology are useful for PWC. These are best employed in a panel. The most useful of these markers include stains for CEA, B72.3, Ber Ep4, CD 15 and MOC-31[39,56]. Identification of neutral mucin with PAS diastase staining is also useful.

Calretinin is a useful marker for mesothelial cells[57]. A small proportion of carcinomas, such a poorly differentiated colonic adenocarcinomas, express calretinin, but most carcinomas are negative[39,58,59]. Even though the ovarian surface epithelium is calretinin positive, less than 10% of serous carcinomas are focally positive for calretinin[39]. Wieczorek and Krane[60] studied the value of calretinin in distinguishing serous borderline tumours from reactive

mesothelial cells and concluded that it was not useful. This was because of the frequent presence of admixed reactive mesothelial cells that will stain positive, and due to the variable staining of both borderline tumours and mesothelial cells. They reported that strips of benign mesothelial cells in PW specimens stain weakly or not at all for calretinin, whereas reactive and malignant mesothelial cells stain strongly. Serous tumours were usually negative but may show focal positivity with granular cytoplasmic staining, whereas mesothelial cells have nuclear and cytoplasmic staining, often more intense on the membrane, producing the characteristic 'fried egg' appearance[61]. Cytokeratin 5/6 staining is another marker of mesothelial differentiation[39] that appears more consistently expressed in normal mesothelial cells and is probably best used in a panel with calretinin and epithelial markers.

Summary

Detection of tumours in PWC relies on the identification of non-mesothelial arrangements and cells. High grade carcinoma is easily identified. However difficulties arise with groups of cells that are not of typical mesothelial nature, particularly when there is an absence of single cells or marked nuclear atypia but raised N/C ratio and small to prominent nucleoli (with or without psammoma bodies). This raises the differential diagnosis of reactive mesothelial cells, endosalpingiosis, borderline serous tumour, low grade serous carcinoma. The morphology of these conditions shows considerable overlap and close attention to clinical history is essential.

Features favouring benign include: cilia, no single cells, few cells with cytoplasmic vacuolation, absence of mitotic activity and two distinct populations not identified.

In cases with equivocal cytological findings correlation with surgical specimens is an essential step. [2,16,48,61]

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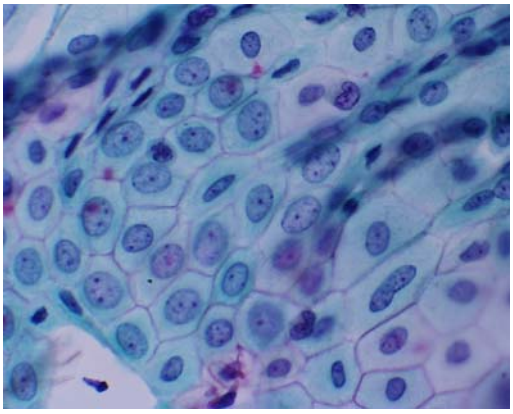


Figure 1. A sheet of benign mesothelial cells arranged in a mosaic pattern with distinct cytoplasmic borders. (Pap; x40 Obj)

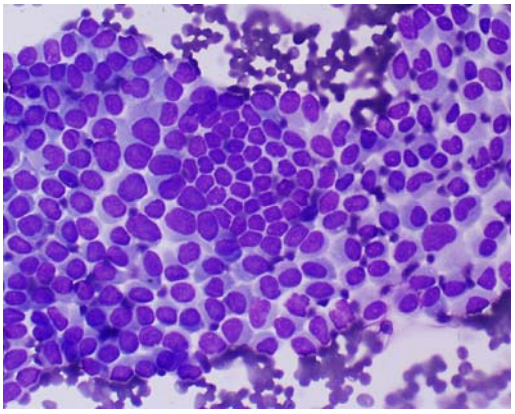


Figure 2. Sheet of mesothelial cells. Note that although variation in nuclear size is apparent throughout the sheet, nuclei are locally uniform. (Diff Quik; x20 Obj)

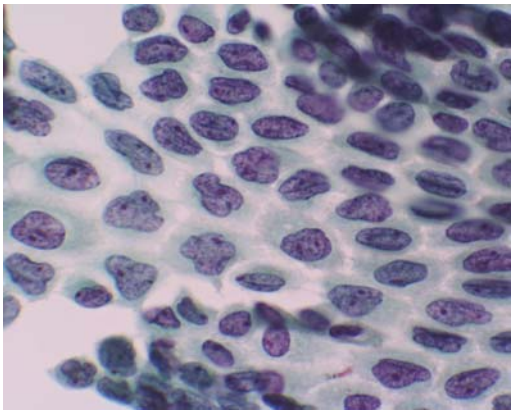


Figure 3. Benign mesothelial cells with highly irregular nuclear membranes and longtitudinal nuclear grooves. (Pap; x40 Obj)

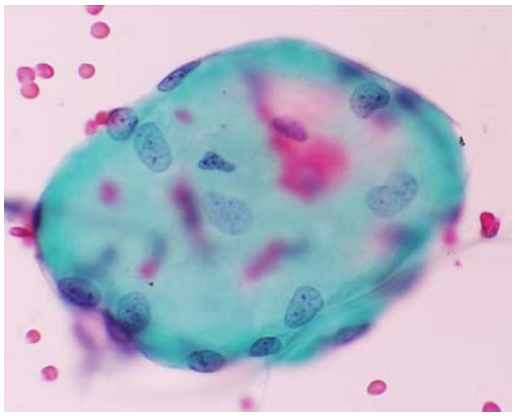


Figure 4. Collagen ball. Note the bland mesothelial cell nuclei arranged around a hyaline globule of collagen. (Pap; x40)

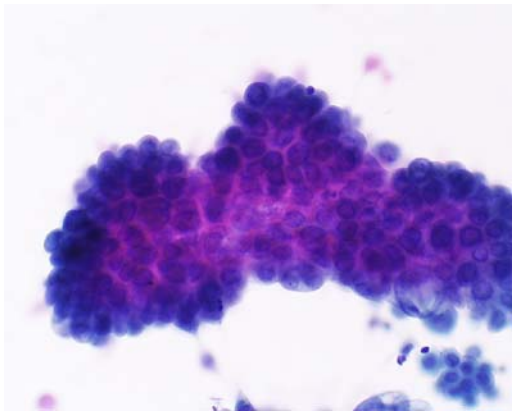


Figure 5. A grouping of cells from an ovarian serous borderline tumour. Note the uniform hyperchromatic nuclei and high N/C ratio. (Pap; x20)

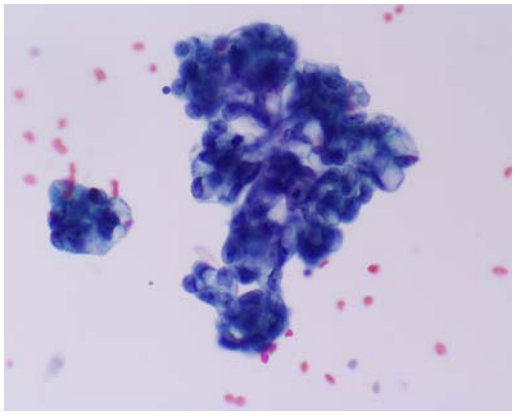


Figure 6. Cell grouping from serous papillary carcinoma of ovary. Note irregular outline of group and cytoplasmic vacuolation. (Pap; x20)

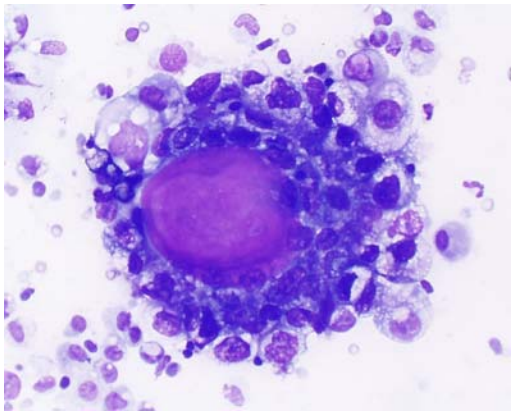


Figure 7. Clear cell carcinoma of ovary. Note cells with abundant finely vacuolated cytoplasm surrounding metachromatic material. (Pap; x20 Obj)

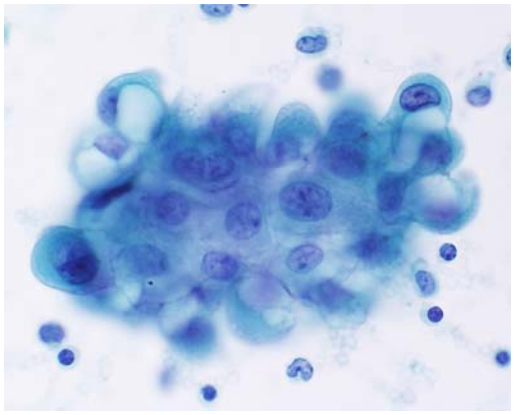


Figure 8. Endometrial adenocarcinoma. Cohesive 3-dimensional grouping of cells with large cytoplasmic vacuoles. (Pap; x40 Obj)

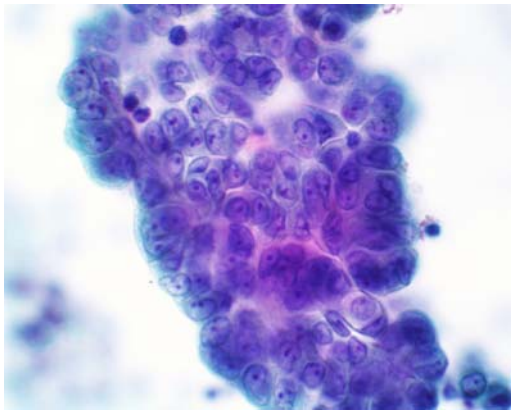


Figure 9. Reactive mesothelial cells with high N/C ratio but forming a monolayered sheet. (Pap, x40 Obj)

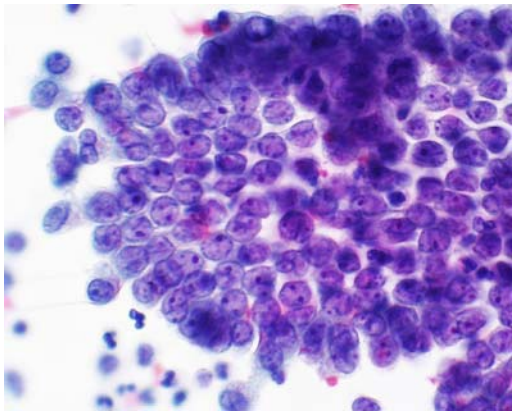
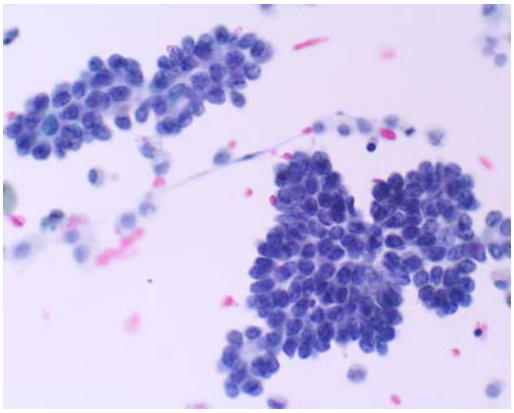
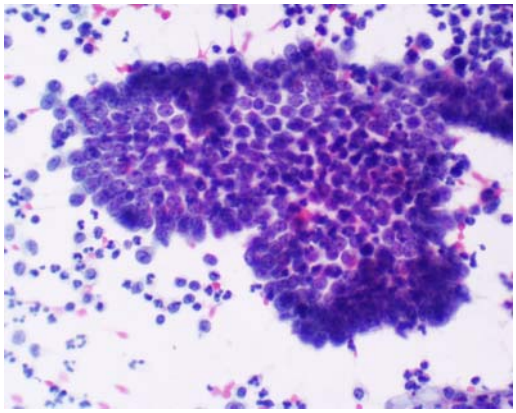


Figure 10. Sheet of reactive mesothelial cells. Note ‘windows’ between cells and monolayered arrangement. (Pap stain, x40 Obj)





A B

Figure 11. Morphological similarity between low grade serous papillary carcinoma of ovary (A) and reactive mesothelial cells (B) from a benign inflammatory specimen. (both x20 Obj).

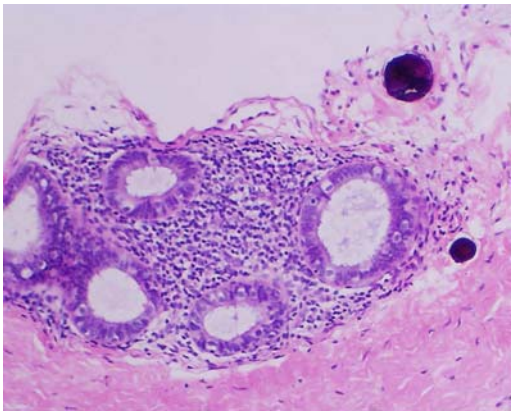


Figure 12. Focus of peritoneal endometriosis with endometrial epithelial and stromal tissue and calcific concretions. (H&E, x10 Obj)

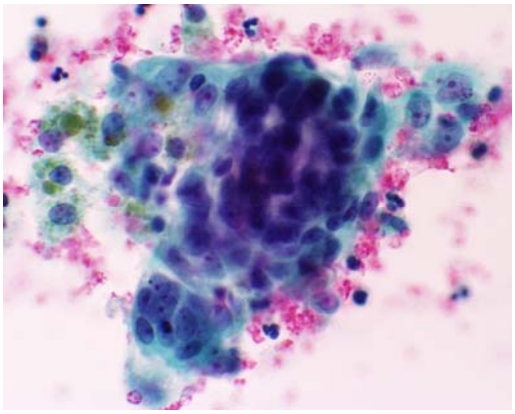


Figure 13. Peritoneal washing from the same patient as Fig 11, showing reactive mesothelial cells and haemosiderophages. (Pap, x40 Obj)

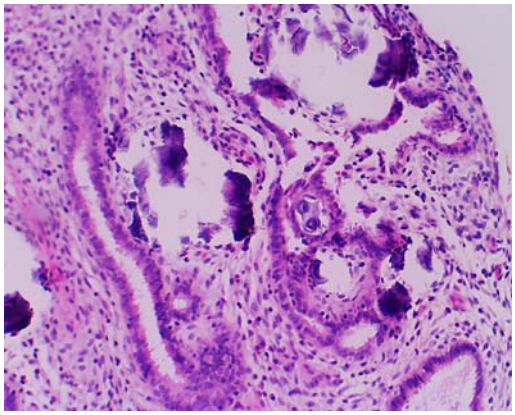


Figure 14. Focus of endosalpingiois in a peritoneal biopsy. Note benign columnar epithelium and calcific concretions. (H&E, x10 Obj)

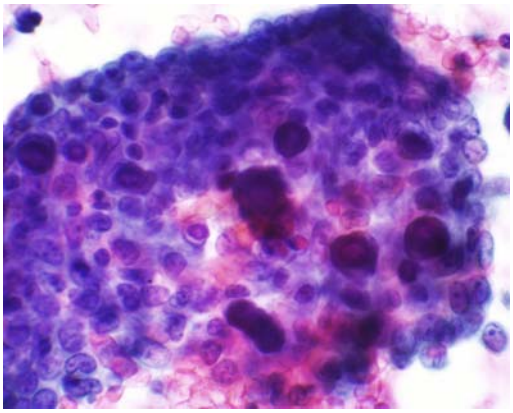


Figure 15. Peritoneal washing from the same case as Fig 14. Note large sheet of cells and psammoma bodies. (Pap stain, x40 Obj)

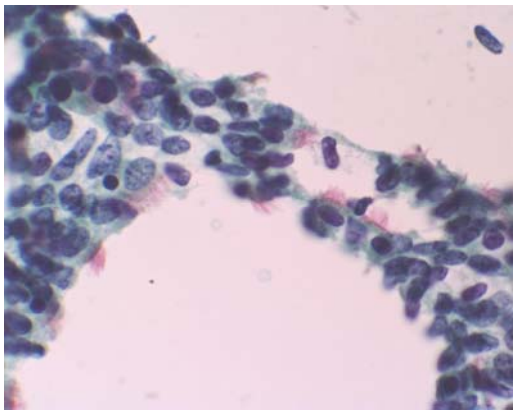


Figure 16. Ciliated epithelial cells in a peritoneal washing from a patient with endosalpingiosis. (Pap, x40 Obj).